

Familial scaphocephaly syndrome caused by a novel mutation in the FGFR2 tyrosine kinase domain

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Craniosynostosis, the premature fusion of one or more sutures of the skull, is a common craniofacial anomaly, with an estimated incidence of 1/2000 to 1/3000 births.^{1,2} Characteristic deformities of skull shape occur as a result of different patterns of sutural fusion, while compensatory skull expansion occurs at unaffected sutures to accommodate the growing brain. Premature fusion of the sagittal suture, for example, results in anterior to posterior elongation of the skull known as scaphocephaly.² Sagittal synostosis is the most common type of craniosynostosis, occurring in 40–58% of cases reported in large neurosurgical surveys, and shows a male predominance.^{3–6} Most of the cases in these reports are “non-syndromic” instances of sagittal synostosis, while familial cases represent 2–9% of the total. Mental retardation is uncommon in isolated sagittal synostosis, but is more common in cases with associated malformations.^{4–6} Over 90 syndromic forms of craniosynostosis have been described. Apert, Crouzon, Pfeiffer, Muenke, and Saethre-Chotzen syndromes are the most common of these, being classified according to the clinical involvement of the cranium, face, hands and feet, and other organs.² In these syndromes, brachycephaly or acrocephaly resulting from coronal synostosis is the predominant cranial deformity, and this is variably associated with mid-face hypoplasia, broad toes, syndactyly, and intellectual deficits. Sagittal synostosis is seen almost exclusively in combination with coronal synostosis,² while macrocephaly in the absence of craniosynostosis occurs infrequently.⁷ Familial scaphocephaly syndromes, which include Berant syndrome, “Philadelphia” type craniosynostosis, and Jones craniosynostosis,² are rare and their molecular aetiology remains unknown.

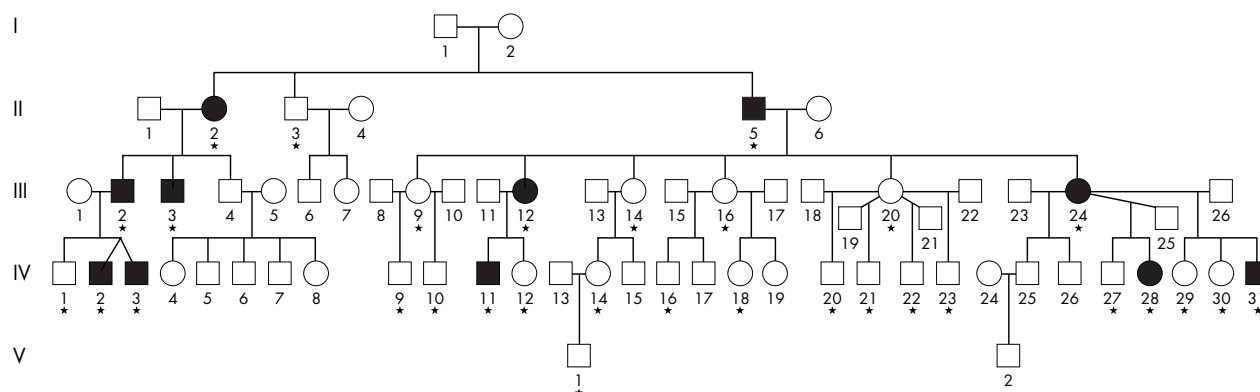
Mutations in five genes have been shown to cause various craniosynostosis syndromes. These are the transcription factor genes *TWIST* (Saethre-Chotzen syndrome, MIM 101400) and *MSX2* (Boston-type craniosynostosis, MIM 604757), and three fibroblast growth factor receptor genes, *FGFR1*, *FGFR2*, and *FGFR3* (Pfeiffer syndrome, MIM 101600; Apert syndrome MIM 101200; Crouzon syndrome, MIM 123500; Jackson-Weiss syndrome, MIM 123500).¹ Mutations in *TWIST*, *FGFR2*, and *FGFR3* also cause isolated non-syndromic coronal synostosis⁸; however, mutations resulting in isolated sagittal suture synostosis and scaphocephaly have yet to be identified. The *FGFR* mutations have been located predominantly in hotspots in these genes in the more than 800 patients where mutations have been characterised.^{1,9} Only one *FGFR1* mutation, P252R, has been identified and it results in a mild Pfeiffer syndrome variant.¹⁰ In contrast, *FGFR2* mutations are a common cause of several craniosynostosis syndromes: Pfeiffer, Apert, Crouzon, Jackson-Weiss, and Beare-Stevenson (MIM 123790).^{1,11} The *FGFR2* mutations are non-randomly distributed throughout the gene, concentrated in exons 8 and 10. Two mutations, S252W and P253R, are relatively common and invariably cause Apert syndrome.^{1,11} In *FGFR3*, the A391E mutation causes Crouzon

Key points

- A large family is reported in which three generations show dominant inheritance of a new craniosynostosis syndrome characterised by scaphocephaly, macrocephaly, severe maxillary retrusion, and mild intellectual disability.
- Molecular analysis revealed a novel K526E mutation in the TK1 portion of the intracellular split tyrosine kinase domain of the fibroblast growth factor receptor 2 gene (*FGFR2*). The mutation is located at the cleft between the TK1 and TK2 lobes of the domain and is predicted to stabilise the activation loop in an active conformation.
- This is the first report of a mutation causing a familial scaphocephaly syndrome with almost exclusive involvement of the sagittal suture. These data indicate that *FGFR2* mutations are responsible for an extended range of phenotypes, which might include isolated sagittal synostosis; they underline the importance of extending *FGFR2* mutation screening beyond the known mutation hotspots.

syndrome with acanthosis nigricans and the P250R mutation causes Muenke syndrome (MIM 602849). Mutations in *FGFR3* also cause thanatophoric dysplasia (MIM 187600). Although craniosynostosis is a feature of this disorder, it is more commonly considered a severe generalised skeletal dysplasia.^{11,12} *FGFR3* mutations also result in a range of other chondrodysplasias including achondroplasia and hypochondroplasia, emphasising the central role that FGF signalling plays in skeletal development.¹² The wide range of limb abnormalities that occurs in the craniosynostosis syndromes indicates that the pathways of cranial bone growth and limb morphogenesis share some critical components. Characterisation of the genes involved in these syndromes is providing important information about the regulatory pathways of craniofacial and skeletal development.

We report the clinical and molecular findings in a three generation family showing autosomal dominant inheritance of a novel craniosynostosis syndrome (fig 1). Thirty family members were examined and the clinical findings are detailed in table 1. The appearances of the 11 affected individuals are shown in fig 2. The study was approved by the Royal Children's Hospital ethics in human research committee, and informed consent was obtained from participating family members. All the subjects shown in fig 2, or their legal guardians, gave written consent for their images to be reproduced in this paper. Macrocephaly was defined as an occipito-frontal circumference greater than the 98th centile



*Individuals who underwent detailed clinical examination

Figure 1 Pedigree of the family affected with a novel scaphocephaly syndrome.

for age.¹³ Hypertelorism was defined as an interpupillary distance greater than the 97th centile for age.¹⁴ Intellectual disability was determined by formal neuropsychological testing in individual IV:11 (Wechsler intelligence scale for children), and by paediatric assessment of individuals IV:2 and IV:31. Individual III:3 did not succeed at mainstream schooling and at 36 years of age was unable to live independently. He was thus considered to have an intellectual disability. Learning difficulty in other individuals was based on family reporting of schooling difficulties. Statistical analysis of the relation between genotype and learning difficulties was carried out using Pearson's χ^2 test.

The 11 affected individuals have varying degrees of craniofacial dysmorphism (fig 1, table 1). Seven (63%) have scaphocephaly and 10 (91%) have macrocephaly. Of 19 unaffected individuals, three had macrocephaly with tall stature, and none had scaphocephaly. More severely affected individuals (for example, IV:11 and III:3) are male and have scaphocephaly, macrocephaly, a high forehead, marked mid-face hypoplasia with severe maxillary retrusion, relative or absolute prognathism, and class III malocclusion. This results in a "scooped out" or concave profile. More severely affected individuals have intellectual disability in the borderline to mild range.

Proband IV:11 presented aged four years with scaphocephaly, macrocephaly, and severe maxillary retrusion, and he has "borderline" intellectual ability (WISC-III). Sagittal

synostosis was confirmed radiologically. He had surgery at 10 and 15 years of age (Le Forte III and maxillary distraction osteotomy, respectively) to advance his midface, improve his dental occlusion, and for cosmetic reasons.

The only other family member who underwent craniofacial surgery was individual IV:31. He had congenital trigonocephaly from metopic synostosis, congenital macrocephaly with macrosomia, broad and medially deviated great toes, complete soft tissue syndactyly of the second and third toes, mild "dysplastic" cerebral ventriculomegaly, and mild developmental delay. He had a bilateral surgical fronto-orbital advancement to correct the metopic synostosis at eight months for cosmetic reasons. His mother (III:24) has a broad great toe and partial 2,3 syndactyly of the toes. Radiographs of the feet of IV:31 and III:24 confirmed the clinical findings, but did not show bony fusion.

Aside from IV:31 and III:24, none of the family members had significant feet findings on examination, and none had hand abnormalities. Mildly affected individuals (for example, II:2) have macrocephaly with a high forehead and mild maxillary retrusion. Hypertelorism appeared to be proportional to the macrocephaly. Affected individuals have variable stature ranging from the 3rd to above than the 97th centile for age.

The clinical presentation of this family thus appears distinct from other known craniosynostosis syndromes. Sagittal synostosis in the absence of coronal synostosis has

Table 1 Summary of clinical findings in 11 affected family members

Characteristic	II:2	II:5	III:2	III:3	III:12	III:24	IV:2	IV:3	IV:11	IV:28	IV:31
Scaphocephaly	-	+	-	+	-	+	+	+	+	+	-*
Macrocephaly	+	+	+	+	+	+	+	+	+	+	+
Head circumference†	58.5	63.5	61	63	59	58	56	57.8	59	60	55.5
98th centile	57.6	58.2	58.2	58.2	57.6	57.6	57.2	57.2	57.8	56.2	52.5
High forehead	+	-	+	-	+	+	+	+	+	+	+
Hypertelorism‡	-	+	+	+	+	+	+	+	+	+	+
Maxillary retrusion	+	+	+	+	+	+	+	+	+	+	+
Malocclusion	-	+	-	+	+	-	-	+	+	+	+
Prognathism§	-	-	+	+	+	-	-	-	-	-	-
High arched palate	+	-	+	-	+	-	+	+	+	-	+
Syndactyly¶	-	-	-	-	-	+	-	-	-	+	++
Broad great toe	-	-	-	-	-	+	-	-	-	-	+
Learning difficulty**	-	+	+	++	-	-	++	+	++	+	++

*Trigonocephaly.

†Head circumference in cm; standard head circumference and percentile values are those described in reference 13.

‡Proportional to macrocephaly.

§Absolute prognathism.

¶2,3 Soft tissue toe syndactyly; +, partial; ++, complete.

**+, family reporting schooling difficulties; ++, borderline or mild intellectual disability or developmental delay on clinical assessment (see text).

ND, not determined; +, present; -, absent.



Figure 2 Appearances of affected family members. Photographs of the 11 affected family members are shown. Lateral photographs of individuals IV:11, II:5 and III:3 show the "scooped out" profile resulting from the severe maxillary retrusion. The three dimensional craniofacial computed tomography scans of individual IV:11 show the patent coronal and lamboid sutures, and the complete obliteration of the sagittal suture. Individual IV:31 is shown before and after craniofacial surgery and the photograph of his feet illustrates the complete 2,3 soft tissue toe syndactyly and the broad great toe. Written consent was obtained from patients or legal guardians for publication of these clinical photographs.

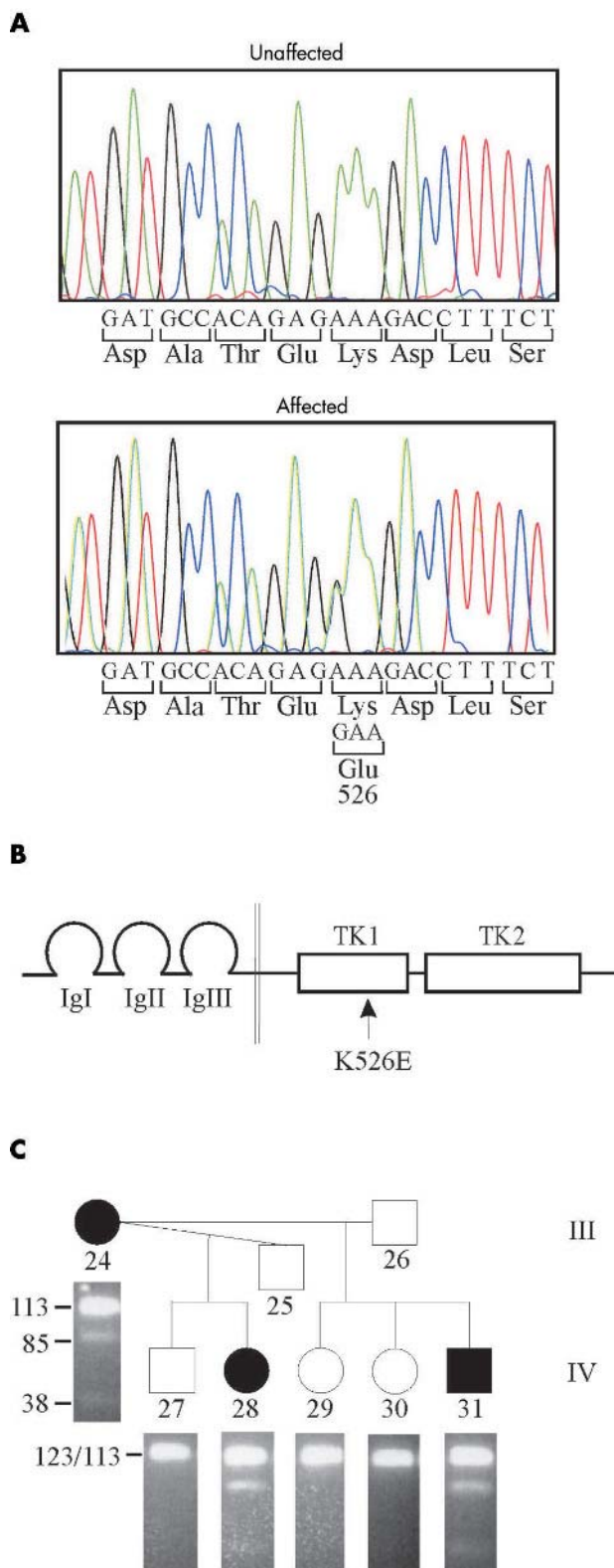


Figure 3 A mutation in the *FGFR2* tyrosine kinase domain. (A) A heterozygous A→G substitution in *FGFR2* exon 14 that resulted in a K526E amino acid substitution was identified in affected family members (lower chromatogram). Protein translations of the first eight complete codons of exon 14 are shown below the chromatogram. This mutation was not present in unaffected family members or controls (upper chromatogram). (B) The novel K526E mutation (arrow) is in the TK1 portion of the split tyrosine kinase domain. The *FGFR2* extracellular immunoglobulin-like domains, IgI, IgII, and IgIII are shown, and the cell membrane is indicated by the two vertical lines. The intracellular split

been reported in Crouzon syndrome in a small percentage of cases, and usually in combination with lambdoid synostosis.² The absence of shallow orbits and ocular proptosis in members of this kindred excludes Crouzon syndrome. Similarly, Pfeiffer syndrome is distinguished by the presence of broad thumbs, and this feature was not present in any affected family members.

A significant proportion of affected individuals had a history of learning difficulties when compared with unaffected family members ($\chi^2 = 9.7$, $p = 0.002$). This difference remained significant after exclusion of the probandi. Mild intellectual disability was associated with a more severe craniofacial phenotype in III:3, IV:2, IV:11, and IV:31. The absence of intracranial hypertension in scaphocephalic family members and the associated facial malformation indicates that intellectual impairment might be a primary consequence of the gene mutation rather than a secondary effect related to timing of surgery.

Initially, we screened for mutations in the genes known to be associated with craniosynostosis. The *FGFR1* P252R mutation found in mild Pfeiffer syndrome, and the Muenke syndrome *FGFR3* P250R mutation¹ were not detected in patient IV:11 by diagnostic restriction enzyme digestion of genomic polymerase chain reaction (PCR) products. The *FGFR2* mutation hot spots in exons 8, 10, and 11 identified in Apert, Crouzon, Pfeiffer, and Beare–Stevenson syndromes,⁹ and the single coding exon of *TWIST* which contains mutations in Saethre–Chotzen syndrome patients,¹ were screened by denaturing high performance liquid chromatography (DHPLC) and gene sequencing. No mutations were found. An activating mutation in the homeobox gene *MSX2* causes dominantly inherited Boston-type craniosynostosis¹⁵ and so we directly sequenced the two exons of *MSX2*. We also sequenced the *ALX4* gene, haploinsufficiency of which results in parietal foramina.¹⁶ No mutations were found in either of these genes.

While most of the characterised *FGFR2* mutations are located in exons 8 and 10 which code for the third extracellular immunoglobulin-like domain (IgIII), or in exon 11 which encodes the transmembrane domain, a recent complete *FGFR2* mutation screen identified mutations in six additional exons—3, 5, 14, 15, 16, and 17.⁹ We therefore screened these exons by DHPLC of genomic PCR products and direct sequencing.

A heterozygous base change, c.1576A→G, was identified in exon 14 of *FGFR2* in patient IV:11 (fig 3A). This is a novel change that results in a non-conservative amino acid substitution, K526E, within the intracellular TK1 domain (fig 3B). The base change creates a new *Mbo*II restriction enzyme recognition site that allowed the development of a diagnostic restriction enzyme assay to track the change in affected and unaffected family members and controls (fig 3C). The change was found in all 11 affected individuals but not in the 19 unaffected family members who were examined, nor in 110 control chromosomes (data not shown), indicating that *FGFR2* K526E is not a common polymorphism and is likely to be the pathogenic mutation in this family. Consistent with this, a lysine residue is found in

tyrosine kinase domain (TK1 and TK2) is indicated by the two boxes. (C) The presence of the mutation was tracked by restriction enzyme digestion (*Mbo*II) of genomic polymerase chain reaction products from 30 family members. The analysis of six family members is shown. Wild-type genotypes resulted in fragments of 123, 113, and 13 bp (individuals IV:27, IV:29, and IV:30). The 123 and 113 bp bands were not resolved and the 13 bp band is too small to see. Carriers of the mutation have bands of 113, 85, 38, and 13 bp (individuals III:24, IV:28, and IV:31). Eleven affected individuals carried the mutation and it was not present in 19 unaffected family members or in 110 control chromosomes.

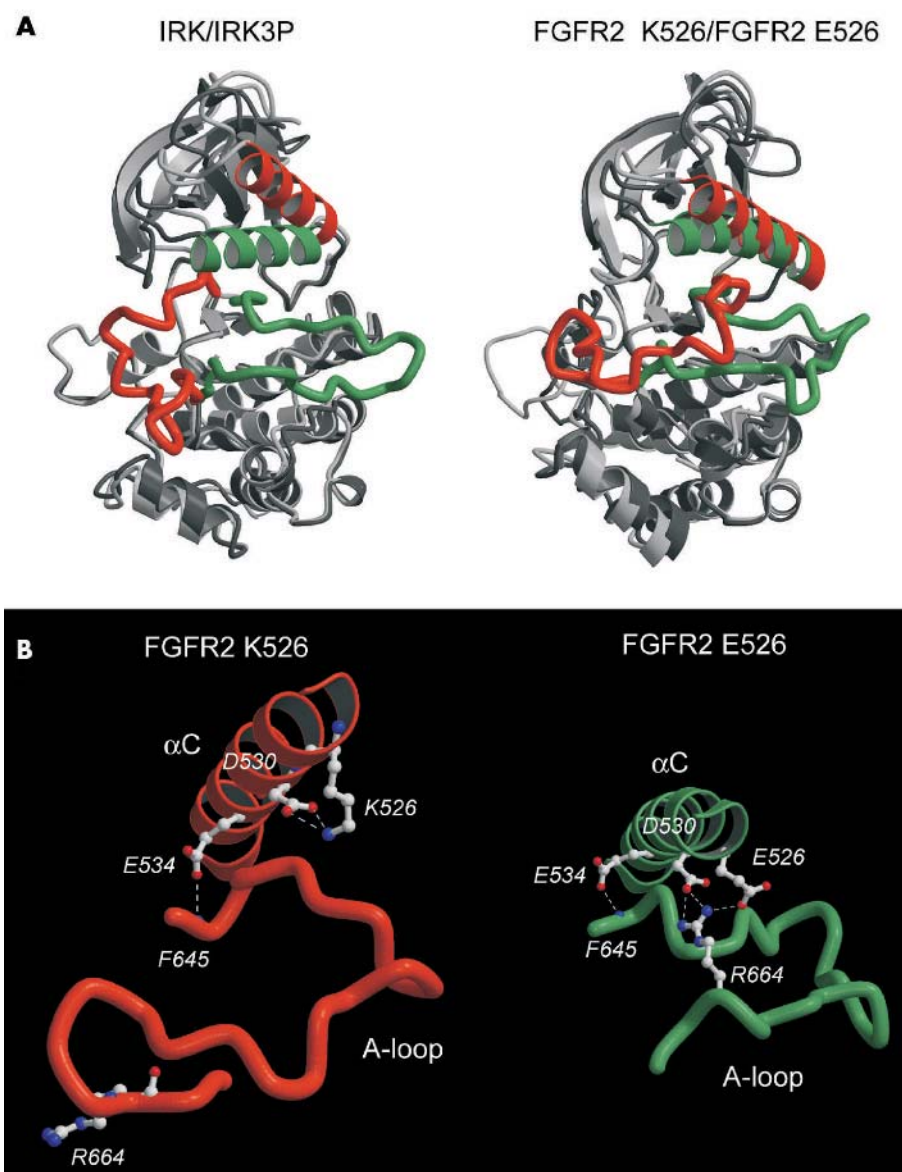


Figure 4 Comparison of the insulin receptor and FGFR2 tyrosine kinase domains. (A) Superimposition of the ribbon diagrams of the insulin receptor tyrosine kinase domain (IRK) coloured light grey, and the activated form of the insulin receptor tyrosine kinase (IRK3P) coloured dark grey, is shown on the left. The α C helix and the activation loop (A loop) of IRK is shown in red and the α C helix and A loop of IRK3P is shown in green. Superimposition of the homology model of the wild-type FGFR2 tyrosine kinase domain (FGFR2 K526, light grey) and the K526E mutant tyrosine kinase (FGFR2 E526, dark grey) is shown on the right. The α C helix and the activation loop (A loop) of the wild-type tyrosine kinase, K526, is shown in red and the α C helix and A loop of the mutant, E526, is shown in green. The conformation of the A loop of the mutant E526 tyrosine kinase resembles the active state seen in IRK3P. (B) The α C helix and activation loop of the inactive wild-type FGFR2 (left) and mutant FGFR2 E526 tyrosine kinase (right). The representations are taken from the superimposed ribbon diagram in (A) and equivalently rotated to more clearly show key interacting residues from the α C helix and A loop. In the wild-type, K526 forms a salt bridge with D530; however, in the mutant the substituted E526 residue and D530 both interact with R664, thereby stabilising the A loop of FGFR2 in an active conformation. The carbon atoms of these residues are indicated in white, oxygen atoms in red, and nitrogen atoms in blue. The hydrogen bond between E534 and the backbone amide hydrogen of F645 is the only other example of bonding between the α C helix and the activation loop. Hydrogens are not shown. The figures were prepared with MOLSCRIPT.²²

the 526 position, or its equivalent, in all FGFR proteins, suggesting that it plays a crucial role in FGFR tyrosine kinase domain function. This is the first report of a pathogenic mutation in a craniosynostosis syndrome manifesting predominantly as scaphocephaly.

To assess the potential pathogenic significance of the mutation we produced structural models of the wild-type (K526) and mutant E526 FGFR2 tyrosine kinase domain (fig 4). The models were based on coordinates from the publicly available crystal structures of the FGFR1¹⁷ (PDB 1FGI) and FGFR2 (PDB 1GJO) tyrosine kinase domains. The

models were produced using the program MODELLER, and refined by conjugate gradient energy minimisation and simulated annealing in explicit water using the program NAMD.^{18–20} All modelling was carried out on the supercomputing facilities at the South Australian Partnership for Advanced Computing (Adelaide, South Australia). Structural comparison with the published structures of the kinase domains from FGFR1,¹⁷ insulin receptor (IRK), and the activated insulin receptor (IRK3P)²¹ confirms that K526 of the wild-type FGFR2 is located in the α C helix at the interface between the N-terminal ATP binding lobe and the C-terminal

substrate binding/catalytic lobe and forms a salt bridge with the adjacent aspartate residue at position 530 (fig 4). Notably, in the model generated for the K526E mutant, the substituted E526 residue and D530 both interact with R664, thereby stabilising the activation loop (A loop) of FGFR2 in an active conformation (fig. 4). Activation of tyrosine kinase domains is accomplished by the ligand dependent autophosphorylation of tyrosine residues in the A loop. This phosphorylation results in a dramatic conformational change in the A loop that permits the binding of ATP and substrate peptide (fig 4A).²¹ Our mutant model predicts that the E526 mutation results in stabilisation of the active conformation of the A loop of the FGFR2 tyrosine kinase domain that probably occurs in the absence of activating ligand. This predicted ligand independent activation will require experimental confirmation.

Six mutations have previously been identified in the intracellular tyrosine kinase domain of *FGFR2*.⁹ Like the K526E mutation described here, all these mutations were located in the cleft between the TK1 and TK2 domains and are thought to activate the tyrosine kinase domain. Two of the mutations K659N and N549H are located at conserved residues equivalent to residues of *FGFR3* at which mutations have been experimentally shown to be activating.^{23–24} The six previous FGFR2 tyrosine kinase domain mutations were identified in patients with syndromic craniosynostosis. Where phenotype data were present, all had either Crouzon syndrome or Pfeiffer syndrome. Some patients had broad thumbs and great toes, and developmental delay was overrepresented compared with typical Crouzon and Pfeiffer syndromes.⁹ One patient in this group, heterozygous for a K641R mutation, had sagittal suture synostosis and scaphocephaly similar to the family described here, but also had broad thumbs with radial angulation and a facial appearance consistent with Pfeiffer syndrome. In contrast, two other Pfeiffer syndrome patients who also carried the K641R mutation did not have scaphocephaly, emphasising the phenotypic variability that is common in craniosynostosis syndromes. There does not appear to be a set of clinical features that uniformly distinguishes patients with FGFR2 intracellular tyrosine kinase mutations from those with mutations in the extracellular region. The current study highlights the importance of extending *FGFR* mutation screening beyond the exon 8 and 10 hotspots. This is particularly important in patients and families with atypical craniosynostosis syndromes. The presence of mutations in the intracellular domains of *FGFR2* should also be considered when sagittal synostosis is found in association with changes in midface development. The significance of *FGFR2* mutations in isolated sagittal synostosis should be further assessed.

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